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Susceptibility of *Aedes aegypti* and *Aedes albopictus* larvae to *Ascogregarina culicis* and *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) from Florida

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Abstract

The susceptibility of *Aedes aegypti* to *Ascogregarina culicis* and *Aedes albopictus* to *Ascogregarina taiwanensis* was examined with mosquito and parasite strains from Tampa, FL. When each host was bioassayed with its natural gregarine, the infection intensity indicated that *Ae. aegypti* was 59% more susceptible to *A. culicis* (87 gamonts/larva) than *Ae. albopictus* to *A. taiwanensis* (47 gamonts/larva). Infections in single and mixed host populations exposed to 100 oocysts/larva of one and both parasites demonstrated that *Ae. aegypti* harbors higher *A. culicis* gamont loads than *Ae. albopictus* of *A. taiwanensis*. In dual gregarine exposures of single host populations, the *A. culicis* infection intensity in *Ae. aegypti* was reduced by ~50%. *A. taiwanensis* exhibited the same capability of infecting *Ae. albopictus* in single and dual exposures. In mixed host populations there were no cross infections, but *A. taiwanensis* in *Ae. albopictus* produced an infection intensity of ~70% lower than that of *A. culicis* in *Ae. aegypti*.

Keywords: Apicomplexa; Gregarine; *Aedes aegypti*; *Aedes albopictus*; *Ascogregarina culicis*; *Ascogregarina taiwanensis*; Strain; Infection intensity; Gamont

1. Introduction

The aseptate gregarines *Ascogregarina culicis* and *Ascogregarina taiwanensis* are host-specific parasites of *Aedes aegypti* and *Aedes albopictus*, respectively (Beier and Craig, 1985), and both are present at high prevalence rates in wild populations of their mosquito hosts in Central Florida (Blackmore et al., 1995). The *Ascogregarina* life cycle occurs almost entirely within the mosquito host. Trophozoite growth and differentiation occur in the larval midgut and gametocytes mature within the Malpighian tubules of pupae and adults. Within the gametocytes, gamete production takes place and fusion produces an oocyst containing eight sporozoites (Chen et al., 1997). Parasite dissemination depends on the ovipositional behavior of infected mosquitoes. Oocysts are expelled by females through the rectum during oviposition in larval habitats, or when either sex of infected adults defecates or dies, releasing oocysts, which are ingested by mosquito larvae (Beier and Craig, 1985). Gregarine pathogenicity varies geographically. Some Asian strains of *A. culicis* are pathogenic to *Ae. aegypti* (Sulaiman, 1992), while the USA strains of *A. culicis* (Barrett, 1968) or *A. taiwanensis* (Fukuda et al., 1997) are considered non-pathogenic to their respective mosquito hosts. However, recent studies have demonstrated that *A. taiwanensis* can exert a detrimental impact upon the fitness of *Ae. albopictus* adults (Comiskey et al., 1999).

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Ae. albopictus has been spreading throughout the United States and the distribution pattern of *Ae. aegypti* has diminished. Before 1985, *Ae. aegypti* was one of the most common and abundant mosquitoes in Florida, but 10 years later, after the establishment of *Ae. albopictus*, the former has been replaced by the latter in most regions of Florida (O'Meara et al., 1995). At the present time, there is no clear explanation for this ecological phenomenon, which is a matter of controversy and has resulted in a number of hypotheses. One of these is the gregarine hypothesis, which is generally stated as "differential pathogenicity of gregarines in interspecies infections may mediate competitive interactions in nature" (Craig, 1993). Apparently, *A. culicis* is a benign parasite for many strains of *Ae. aegypti* but it might have a detrimental impact upon the ability of *Ae. aegypti* adults to confront adverse environmental conditions (Hayes and Haverfield, 1971). Likewise, there is a decrease in the wing-length and fecundity for *Ae. albopictus* females infected with *A. taiwanensis* (Comiskey et al., 1999).

Although both *Aedes* species share the same larval ecological niche, and cross and dual infections occur in nature, the intensity and impact of these infections remain unknown. Most studies have focused on determining *Ascogregarina* spp. infection rates in field populations of the mosquito hosts (Blackmore et al., 1995; Garcia et al., 1994) but only at the genus level because it was only recently that gamont differentiation at the species level in dual-infected larvae could be determined (Reyes-Villanueva et al., 2001). We conducted experiments on basic host/parasite interactions with experimental populations collected from a locality where the competitive displacement process was in progress. The understanding of parasitic relationships is a key requirement to clarify how these sympatric mosquito hosts may interact in nature. Specific objectives of this study were: (1) to determine the susceptibility (gamont load per larva) of two strains of *Ae. aegypti* and *Ae. albopictus* (wild, Tampa and laboratory, USDA strain) to *A. culicis* and *A. taiwanensis* and (2) to evaluate the gamont intensity of each gregarine species when *Ae. aegypti* and *Ae. albopictus* larvae (mixed) were exposed to one of the gregarine species or both species simultaneously.

2. Material and methods

Wild larvae and pupae of *Aedes* spp. were collected from cemetery flowerpots in Tampa, FL 1999, returned to the laboratory and placed in a cage for emergence. Male and female adults of *Aedes aegypti* and *Ae. albopictus* were transferred to separate cages and provided 10% sucrose solution on cotton pads over four days to allow for mating. After four days, adults were allowed to feed on a guinea pig. A day after blood feeding, engorged females were individually placed into screen capped vials (50 ml) with ~10-ml distilled H_2O plus a small piece of paper for oviposition. After eggs were laid, adults were classified as healthy and infected by the presence of gametocytes with mature oocysts inside the lumen of Malpighian tubules, which were dissected from females in 5% Ringer's solution and observed with a phase-contrast microscope. Thus, a healthy and an infected colony of each *Aedes* species with its own gregarine were established to conduct the susceptibility bioassays of each host–gregarine system of the wild strains from Tampa. To examine differences in susceptibility that either *Ae. aegypti* or *Ae. albopictus* have to their gregarines in nature, we established a laboratory strain of each mosquito species from colonies maintained at the Center for Medical, Agricultural, and Veterinary Entomology (CEMAVE) from the USDA at Gainesville, FL. Wild (Tampa strain) as well as the insectary strain (USDA strain) of *Ae. aegypti* and *Ae. albopictus* were bioassayed by exposing them to oocysts of *Ascogregarina culicis* and *A. taiwanensis*, respectively, which were originally isolated from the Tampa strain of both mosquito species.

Two experiments (one each for *Ae. aegypti* and *Ae. albopictus*) and two bioassays (the two strains of each mosquito species) per experiment were conducted. Each bioassay consisted of six treatments (five oocyst concentrations plus control), replicated three times. Oocysts used in these experiments were harvested from the infected colony of each *Aedes* species by macerating in 5-ml distilled H_2O approximately 250 infected adult mosquitoes of each species with a tissue homogenizer and then filtering through wet cotton in a glass syringe. The crude extract was centrifuged at 5000 rpm for 10 min and oocyst concentrations determined with a hemocytometer and a phase-contrast microscope. A stock suspension of 10,000 oocysts/ml was prepared and oocyst concentrations of 6, 12, 25, 50, and 100 oocysts/larva, plus a control, were established. Each treatment consisted of a group of 100 second-instars placed in 100-ml distilled H_2O in a 3.5-oz plastic cup with the oocysts and exposed for 24 h. A small amount (0.04 g) of artificial larval diet, which was 5% alfalfa and potbelly pig chow mixture (2:1), was added to the test cups to promote oocyst ingestion and larval infection. Control cups were set up at the same time except that oocysts were not added to the water. After 24-h exposure, the contents of each cup including water, oocysts, and larvae were poured into an enamel pan containing 1 liter distilled H_2O. Controls also were decanted to enamel pans, and both test and control pans were placed in an insectary held at 27 °C, 80% RH, and 14:10 h (D:L), where larvae were held throughout the remainder of their development. Three milliliters of 5% larval diet (described above) was added to each pan at approximately 48 h (second day) after hatching and then 5 ml of the same

suspension were added to each pan at approximately 120 h (fifth day) post-hatching.

To determine the number of gamonts in larvae, ~6 h before pupation 10 larvae were taken from each pan and midguts dissected in 5% Ringer's solution under a dissecting microscope at 16× magnification. The midgut of each larva was pulled from the body with sharp forceps after making two cuts: the first cut removed the head and the second cut was made at the middle of the last abdominal segment. Once the midgut was removed intact, accurate counts were made of all the gamonts present in the lumen between the epithelium and the peritrophic matrix of each specimen. The infection intensity was determined and expressed as gamont density/larva for the "Tampa strain" and "USDA strain" of *Ae. aegypti* exposed to oocyst concentrations (treatments) of 6, 12, 25, 50, and 100 oocysts/larva of *A. culicis* plus the control in the first experiment. The same was done in the second experiment for both strains of *Ae. albopictus* to *A. taiwanensis*. Dead larvae in all treatments were recorded.

3. Experiments with mixed larval treatments exposed to one or both gregarines

A stock suspension of 1000 oocysts/ml of each gregarine sp. was prepared by macerating infected adult mosquitoes in distilled H_2O with a tissue homogenizer. The homogenate was filtered to eliminate host debris, and oocysts were counted on a hemocytometer. Groups of 100, 48-h-old healthy *Ae. aegypti* and *Ae. albopictus* larvae were exposed to the oocysts in 3.5-oz plastic cups in 100 ml of water plus a small amount of artificial diet as above. Groups without the addition of the gregarine served as controls. After an exposure of 24 h, the contents of each cup including water, oocysts and larvae were poured into an enamel pan containing 500 ml of distilled H_2O and placed into an insectary held at 27 °C, 80% RH, and 14:10 h (D:L). Larval diet was the same and it was provided as above.

Larval guts were dissected as described above. Three experiments were performed. In the first experiment, the density of gamonts/larva for each gregarine per mosquito host species was determined in mixed larval treatments (50 *Ae. aegypti* + 50 *Ae. albopictus* larvae) that were singly and dually infected with *A. culicis* and/or *A. taiwanensis*. The second and third experiment determined the single and cross-infection rates of both gregarines (100 oocysts/larva) in *Ae. aegypti* and *Ae. albopictus* larvae.

4. Statistical analysis

The susceptibility of the two strains of each *Aedes* species to the infection of its corresponding *Ascogregarina* species was determined by linear regression between gamont density/larva as the dependent variable and oocyst concentration/larva as independent variable. The regression equation, Pearson correlation coefficient, and ANOVAs were computed by using the SAS procedures PROC REG and PROC GLM, which included pair wise REGWQ tests. Means per treatment, strain, and replicate were calculated with the SAS procedure PROC MEANS, while PROC UNIVARIATE procedure was used to check data normalization (SAS Institute, 1988).

5. Results

5.1. Bioassays with Ae. aegypti infected with A. culicis and Ae. albopictus infected with A. taiwanensis

In general, the mean number of gamonts/larva varied as a function of the mosquito host ($F = 23.44$, $P < 0.01$), mosquito strain ($F = 23.21$, $P < 0.01$), and dose (oocyst concentration) ($F = 133.05$, $P < 0.01$). In the analysis performed for each mosquito host, the mean number of *A. culicis* gamonts in *Ae. aegypti* larvae varied depending on the strain ($F = 50.94$, $P < 0.01$) and dose ($F = 114.61$, $P < 0.01$). In contrast the same analysis for the *A. taiwanensis* in *Ae. albopictus* revealed that the mean number of gamonts/larva did not vary by strain ($F = 0.91$, $P = 0.34$) but did differ by dose ($F = 49.88$, $P < 0.01$).

The *A. culicis* gamont intensity in the larval midgut of the *Ae. aegypti* "Tampa strain" had an overall average of 87 ± 9 gamonts/larva, which was significantly higher than the mean of 53 ± 9 gamonts/larva of the "USDA strain." In contrast, in the *Ae. albopictus* "Tampa strain" the *A. taiwanensis* gamont intensity averaged 53 ± 16 gamonts/larva, which was not significantly different from the 47 ± 9 gamonts/larva found for the "USDA strain" (Table 1). The higher intensity of infection for *A. culicis* in *Ae. aegypti* also was reflected in the larval infection rate. In *Ae. aegypti*, the average larval infection rate for the "Tampa strain" and "USDA strain" were 99 and 96%, whereas the infection rate of *A. taiwanensis* for both *Ae. albopictus* strains was 88 and 90%, respectively (Table 1).

As expected, the higher the oocyst concentration, the higher the gamont intensity/larva in both host/parasite systems, and the correlation between both variables was high in both systems. However, the gamont intensity always tended to be higher in the "Tampa strain" of *Ae. aegypti* (Figs. 1 and 2).

The difference in susceptibility for each mosquito species infected with its own gregarine was more obvious in the linear regression of the gamont density/larva versus oocyst concentration of the "Tampa strain" for both mosquito hosts. The increments in gamont intensity of *A. culicis* as a function of the oocyst dose were higher in *Ae. aegypti* than those of *A. taiwanensis* in *Ae. albopictus*. The slope values in both linear regression equations were 1.98 and 1.49, respectively (Figs. 1 and 2).

Table 1
Infection intensity expressed as mean gamonts/larva[a] in two strains of *Aedes aegypti* exposed to five oocyst concentrations of *Ascogregarina culicis*, and two strains of *Ae. albopictus* exposed to five oocyst concentrations of *A. taiwanensis*

Species	Strain	Oocyst dose	Mean gamonts/larva ± SE[b]	Infection (%)[c]	Mortality (%)[d]
Ae. aegypti	Tampa	6	19 ± 2	100	3.5
	Tampa	12	39 ± 6	100	4.0
	Tampa	25	70 ± 2	100	3.9
	Tampa	50	92 ± 18	97	7.1
	Tampa	100	215 ± 7	100	7.3
Average			87 ± 9**a**	99	5.2
	USDA	6	17 ± 2	87	5.3
	USDA	12	23 ± 4	93	6.4
	USDA	25	42 ± 12	100	3.2
	USDA	50	74 ± 20	100	4.7
	USDA	100	108 ± 14	100	8.3
Average			53 ± 9**b**	96	5.6
Ae. Albopictus	Tampa	6	8 ± 1	70	4.2
	Tampa	12	16 ± 3	83	4.6
	Tampa	25	40 ± 15	90	4.9
	Tampa	50	51 ± 15	100	5.4
	Tampa	100	150 ± 33	97	5.3
Average			53 ± 16**a**	88	4.9
	USDA	6	10 ± 4	77	4.1
	USDA	12	17 ± 0	90	4.5
	USDA	25	35 ± 15	90	7.2
	USDA	50	53 ± 6	97	8.3
	USDA	100	118 ± 17	97	12.6
Average			47 ± 9**a**	90	7.3

[a] Mean gamonts/larva was calculated from $n = 30$ larvae.
[b] SE, standard error.
[c] Infection (%), infected larvae in 30 dissected larvae. The average mean gamonts/larva per strain per species, with different letters are significantly different according to a REGWQ test ($P < 0.05$) (SAS Institute, 1988).
[d] Mortality, average per replicate (sum of dead insects in three replicates/3). A replicate, number of dead insects in a 90-larvae set. The averages of mortality/replicate were not significantly different according to a Kruskal–Wallis test ($\chi^2 = 0.57$).

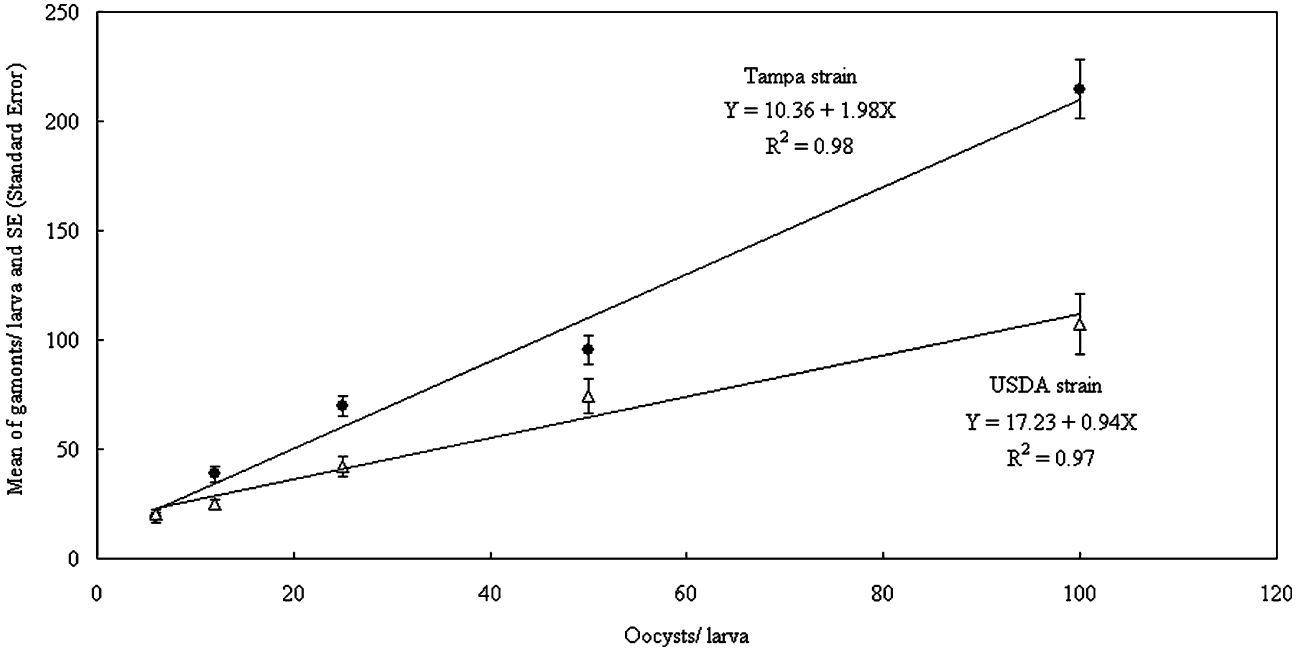

Fig. 1. Susceptibility of the Tampa (upper line) and USDA (lower line) strains of *Aedes aegypti* to *Ascogregarina culicis* exposed to five oocyst concentrations and expressed in gamonts/larva ± SE (vertical line).

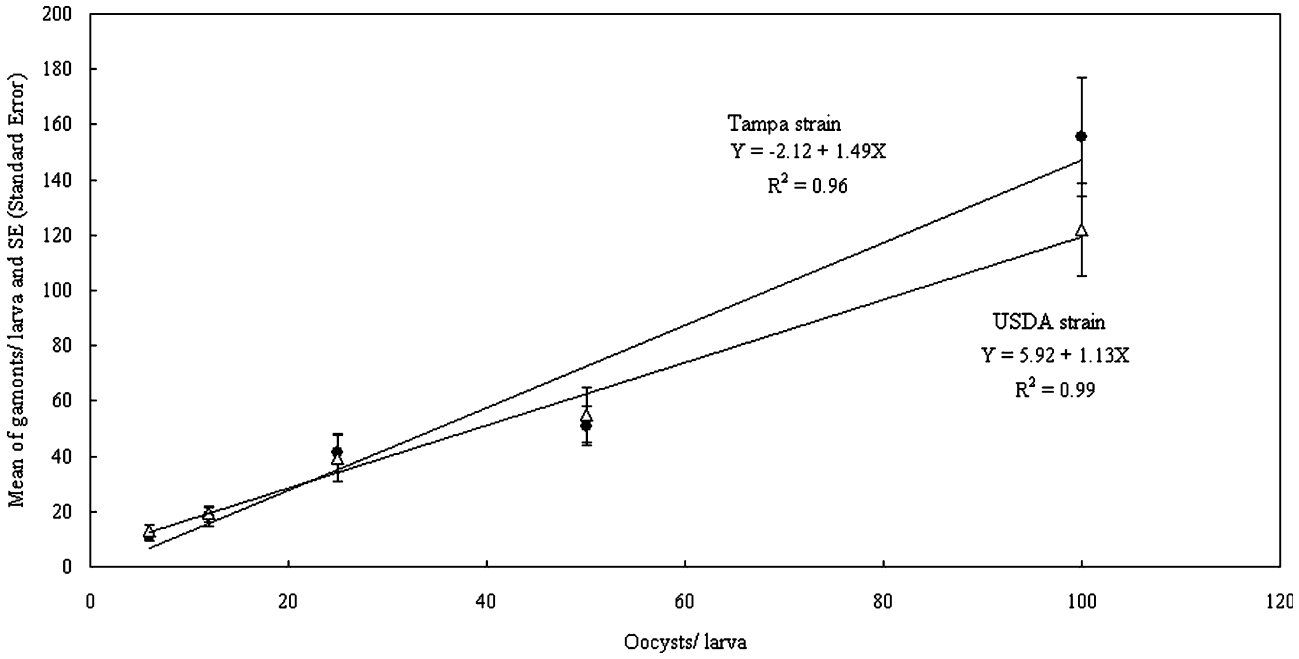

Fig. 2. Susceptibility of the Tampa (upper line) and USDA (lower line) strains of *Aedes albopictus* to *Ascogregarina taiwanensis* exposed to five oocyst concentrations and expressed in gamonts/larva ± SE (vertical line).

5.2. Gamont density of *A. culicis* and *A. taiwanensis* in single and dual exposures of *Ae. aegypti* and *Ae. albopictus* larvae

The mean gamont intensity/larva for *A. culicis* and *A. taiwanensis* varied significantly in larval treatments that consisted of one or both *Aedes* species in single and dual exposures (Table 2). Although *A. culicis* and *A. taiwanensis* can infect an alternate host, there was a clear preference for the natural hosts (Table 2). The highest infection intensities of *A. taiwanensis* were found in single (5 gamonts/larva) and dual (2 gamonts/larva) exposures made with populations of *Ae. aegypti*. In contrast, *A. culicis* was able to establish infections only in populations of *Ae. albopictus* exposed to both gregarines (1 gamont/larva).

Table 2
Mean gamont density per larva ± standard error (SE) of *Ascogregarina culicis* and *A. taiwanensis* in fourth instars of *Aedes aegypti* and *Ae. albopictus* single- or dual-infected as 24-h-old larvae at a dose of 100 oocysts/larva of each gregarine

Mosquito host	Exposure	Parasite	Mean ± SE[d]
Ae. aegypti[a]	Single	*A. culicis*	143 ± 15 **a**
Ae. aegypti	Single	*A. taiwanensis*	1 ± 0 **b**
Ae. aegypti	Dual	*A. culicis*	69 ± 10 **c**
Ae. aegypti	Dual	*A. taiwanensis*	0 ± 0 **b**
Ae. albopictus	Single	*A. culicis*	0 **a**
Ae. albopictus	Single	*A. taiwanensis*	46 ± 13 **b**
Ae. albopictus	Dual	*A. culicis*	0 **a**
Ae. albopictus	Dual	*A. taiwanensis*	48 ± 13 **b**
A. aegypti[b]	Single	*A. culicis*	202 ± 15 **a**
A. aegypti	Single	*A. taiwanensis*	5 ± 1 **b**
A. aegypti	Dual	*A. culicis*	104 ± 13 **c**
A. aegypti	Dual	*A. taiwanensis*	2 ± 1 **b**
Ae. albopictus[c]	Single	*A. culicis*	0 **a**
Ae. albopictus	Single	*A. taiwanensis*	87 ± 15 **b**
Ae. albopictus	Dual	*A. culicis*	1 ± 1 **a**
Ae. albopictus	Dual	*A. taiwanensis*	62 ± 13 **b**

$N = 20$ larvae.
[a] Mixed larvae single- and dual-infected.
[b] Only *Ae. aegypti* exposed to single and dual infections.
[c] Only *Ae. albopictus* exposed to single and dual infections.
[d] Means with different letters are significantly different according to a REGWQ test ($P < 0.05$) (SAS Institute, 1988).

The presence of *A. taiwanensis* significantly inhibited the infection intensity of *A. culicis* in *Ae. aegypti* when dual exposures were made in either single or mixed larval populations. As expected, infection intensities of *A. culicis* were greatest (202 gamonts/larva) in single exposures made with only populations of *Ae. aegypti*, but the presence of *A. taiwanensis* significantly reduced the *A. culicis* intensities to 104 gamonts/larva (Table 2). Likewise, in exposures made with mixed mosquito populations, gamont intensities in *Ae. aegypti* were highest with single exposures of *A. culicis* (143 gamonts/larva) and were significantly reduced (69 gamonts/larva) when *A. taiwanensis* was present (Table 2). In contrast, the infection intensities of *A. taiwanensis* in *Ae. albopictus* were not changed (87 gamonts/larva) in single exposures made in populations of *Ae. albopictus* and was not different (62 gamonts/larva) when *A. culicis* was present (Table 2). The lowest infection intensities of *A. taiwanensis* were observed with mixed larval populations in either single (46 gamonts/larva) or dual exposures (48 gamonts/larva).

Finally, the overall mortality rate expressed as the mean combined mortality rate (larvae + pupae) per replicate varied from 3.2 to 12.6% for all the oocyst concentrations tested within each strain per mosquito species. The mean mortality rates were not significantly different between host species and strains (Table 1).

6. Discussion

This is the first study to determine with bioassays the susceptibility of two US strains of *Ae. aegypti* to *A. culicis* and of *Ae. albopictus* to *A. taiwanensis.*

The ''USDA strain'' and the wild ''Tampa strain'' of *Ae. albopictus* were equally susceptible to *A. taiwanensis*. The *Ae. albopictus* ''USDA'' colony was recently established with specimens collected from the Gainesville, FL area when the ''Asian tiger'' was invading Florida in the second part of the 1980s. In contrast, in the *Ae. aegypti*/*A. culicis* combination, the *Ae. aegypti* ''Tampa strain'' displayed a higher susceptibility to *A. culicis* than to the *Ae. aegypti* ''USDA strain'' which suggests that the two strains are genetically distinct. The USDA *Ae. aegypti* colony was established from the ''Orlando strain'' in the 1960s and since that time has not been in contact with the gregarine. Possibly resistant genotypes to the infection of *A. culicis* emerged during the 25 years in colony as a consequence of endogamy and genetic drift.

This study was conducted with experimental populations of both host and parasite collected from a locality where *Ae. aegypti* is being displaced by *Ae. albopictus*. Prior to this report, there has been only one study in Asia (Thailand) by Sulaiman (1992), in which bioassays were used to determine the susceptibility of *Ae. aegypti* to *A. culicis*. Sulaiman (1992) concluded that the gregarine pathogenicity depends on both strain and genetic compatibility between the gregarine and geographic strains of mosquito host.

Mortality observed in this study confirms the reports in the literature that the American strains of both *Ascogregarina* species are benign parasites in their natural hosts (Beier and Craig, 1985; Stapp and Casten, 1971). Although the first report for the presence of *A. culicis* in the USA stated a significant mortality in *Ae. aegypti*, mortality data were not provided in the paper (Barrett, 1968). In the case of *A. taiwanensis* larval mortality rates of up to 10% have been reported for both *Aedes* species (Garcia et al., 1994), which is in line with the larval–pupal mortality observed here ranging from 3.2 to 12.6% in both host/parasite systems.

Our estimates of the mean infection intensities for the ''Tampa strain''of *A. culicis* in *Ae. aegypti* (87 gamonts/larva) and *A. taiwanensis* in *Ae. albopictus* (53 gamonts/larva) are similar to those reported in the literature. A survey from 12 Florida cities to detect the prevalence of *A. culicis* in *Ae. aegypti* was conducted through the dry and wet seasons of 1968. The infection intensity varied from 1 to over 800 trophozoites/larva with an overall mean of 57 trophozoites/larva (Stapp and Casten, 1971). In another Florida survey, the *Ascogregarina* spp. infection intensity ranged from 1 to 486 trophozoites/larva, and the average was higher in *Ae. aegypti* (53 gamonts/larva) than in *Ae. albopictus* (34 gamonts/larva) (Blackmore et al., 1995). In a survey done in Gainesville, FL, the average highest natural prevalence (65 gamonts/larva) reported for *A. taiwanensis* in *Ae. albopictus* in that locality, was detected in larvae collected from shaded tires (Garcia et al., 1994). Lastly, Sulaiman (1992) reported a gamont intensity of 69 gamonts/larva but with high mortality (>70%) for a dose of 100 oocysts/larva of the Korn Kean strain of *A. culicis*, which is the parasite strain normally found in the *Ae. aegypti* populations in the locality around Bangkok, Thailand. This infection intensity is almost the same that we found here (70 gamonts/larva) when *Ae. aegypti* was infected with only 25 oocysts/larva and with only 3.9% mortality. When we infected larvae (Tampa strain) with 100 oocyst/larva, the mean gamont intensity increased almost four times (215 gamonts/larva) while the mortality was 7.3%, which means that the parasite sporozoites from Florida were capable of invading and developing in the host epithelial cells at a rate four times higher that the rate observed in the *Ae. aegypti* Thailand strains.

The USA strains of *Ae. aegypti* and *Ae. albopictus* are able to tolerate high gamont intensities of *A. culicis* and *A. taiwanensis*, respectively, without significant mortality. However, based on our results, we cannot conclude that these parasites are totally benign for their corresponding mosquito hosts because they could impact host fitness. This was a preliminary study to gather information about basic host/parasite relationships. Further research involving cross and dual infections and

more stressful conditions in mosquito larvae may demonstrate whether these gregarines play a role in the competition between both mosquito hosts and to favor one or another through adult development.

Consistently, under comparable dose and exposure conditions, the gamont densities of *A. culicis* in *Ae. aegypti* were generally two to three times greater than the densities of *A. taiwanensis* found in *Ae. albopictus*. The reason for this reduction is not known but insects are reported to resist midgut infections by desquamation (excretion) of midgut cells into the lumen of the digestive tract (Tanada and Kaya, 1993). Surprisingly, the gamont densities of *A. culicis* in *Ae. aegypti* were reduced by approximately 50% when *A. taiwanensis* was present at the time of exposure. Because *A. taiwanensis* only rarely became established in *Ae. aegypti*, the lower densities of *A. culicis* may result from fewer attachment points in the midgut caused by the loss of cells to which *A. taiwanensis* attached.

Although it has been reported that *A. taiwanensis* is unable to complete its life cycle in hosts other than *Ae. albopictus* (Lien and Levine, 1980), *Ascogregarina* species are not host-specific parasites in mosquitoes. Recently, oocysts recovered from *Ochlerotatus taeniorhynchus* adults that were infected with oocysts extracted from *Ae. albopictus* (presumably *A. taiwanensis*) were infectious for *Ae. aegypti*, *Ae. albopictus*, and *Oc. taeniorhynchus* larvae. (Garcia et al., 1994). This capability of *A. taiwanensis* to complete its life cycle in alternate hosts represents an adaptive strategy for long-term survival. Many basic aspects on the biology of both *A. taiwanensis* and *A. culicis* remain unknown. An early study demonstrated that *A. taiwanensis* was able to infect *Ae. aegypti* larvae at a rate of 48% (Lien and Levine, 1980). In a more recent study, infection rates of 56.3 and 12% in *Ae. aegypti* larvae by *A. taiwanensis* were induced in the laboratory and included the recovery of infective oocysts from *Ae. aegypti* adults (Munstermann and Wesson, 1990). We have conclusively demonstrated that *Ae. aegypti* is more susceptible to *A. culicis* than *Ae. albopictus* is to *A. taiwanensis*. However, this laboratory study does not support the "gregarine hypothesis" proposed by Craig (1993) to explain the *Ae. aegypti* displacement by *Ae. albopictus* in the southeast USA. To investigate how our finding may relate to interactions under natural conditions, we are conducting experiments to determine how *A. culicis* and *A. taiwanensis* may impact the differential survival of sympatric populations of *Ae. aegypti* and *Ae. albopictus* in the field.

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